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Introgression of the low browning trait from the wild Mexican species *Solanum hjertingii* into cultivated potato (*S. tuberosum* L.)

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Summary

Internal discoloration of tubers resulting from impact damage (blackspot bruise) is a serious quality problem in potato production and utilization, reducing profits to growers and increasing costs for processors. Resistance to blackspot bruise has been identified in the wild species *Solanum hjertingii* and is therefore a potential germplasm resource for genetic resistance to this problem. A bridging cross between *S. hjertingii* and a cultivated diploid clone was used to produce a triploid hybrid population that exhibited very low tuber browning potential, indicating a dominant pattern of inheritance for this trait. The triploid progeny were subjected to *in vitro* chromosome doubling and the resulting hexaploid clones were screened for browning potential. A hexaploid clone selected for low browning was reciprocally crossed with cultivated *S. tuberosum* cultivars exhibiting high susceptibility to blackspot bruise. Tubers obtained from the seed progeny of these 4x-6x crosses (hereafter referred to as the BC_1 populations) were evaluated for browning potential and polyphenol oxidase (PPO) activity. Tubers from the BC_1 populations displayed a very low potential for melanin production, while PPO activity was quite variable. The low Pearson correlation coefficient ($r^2 = 0.45$), between browning potential and PPO activity suggests that the mechanism of blackspot bruise resistance derived from *S. hjertingii* cannot be explained simply as a reduction in the initial PPO activity. The expression of substantial resistance to browning and dominant expression pattern in these BC_1 progeny indicate that utilizing genetic elements derived from *S. hjertingii* provides a robust approach for developing blackspot bruise resistant potato varieties.

Abbreviations: PPO – polyphenol oxidase; d/a – degree of dominance; EBN – endosperm balance number; gfw – grams fresh weight

Introduction

Internal discoloration of tuber tissue following impact or compression damage during harvest or storage (referred to as internal bruise, blackspot bruise, or bluespot bruise) presents a significant quality problem in the potato industry. Bruising reduces profits to growers through lost premiums, increases the sorting and culling costs for processors and presents a serious consumer satisfaction problem in fresh market potatoes. Control of bruising losses in the commercial potato industry currently consists of close attention to cultural practices during tuber growth combined with careful handling procedures during harvest and storage. Although some differences in resistance to bruising between various potato genotypes have been noted, many cultivars and breeding lines with desirable agricultural and quality traits are highly susceptible to bruise. Minimizing bruise losses in bruise-prone cultivars requires attention to maturity at harvest (Corsini et al., 1999), the water potential of tubers at harvest and in storage (McGarry et al., 1996), nutritional status, (particularly potassium, calcium and nitrogen levels (Brook, 1996; Storey & Davies, 1992; Kleinhenz et al., 1999)), and careful handling to avoid impact damage during harvest, transport and storage.

Although difficult to quantify, estimates of the economic impact from bruise losses fall in the range of $10,000 to $50,000 per grower in lost premiums each year (Mathew & Hyde, 1997; USDA, 1994) and up to a $128 million annual cost to the potato industry as a whole (Brook, 1996).

The biochemistry of the browning reaction suggests that reduction of bruise susceptibility can be approached in a number of ways. Numerous studies have demonstrated that tuber browning is the result of mechanical tissue disruption allowing a plastid localized enzyme, polyphenol oxidase (PPO; EC1.10.3.2 or EC1.14.18.1) to come into contact with monophenolic and ortho-diphenolic compounds from the vacuole and cytoplasm (Mayer, 1987; Whitaker & Lee, 1995). The PPO enzyme catalyzes the reaction between a variety of phenolic compounds and molecular oxygen to produce ortho-diquinones. These highly reactive diquinones then react spontaneously and nonspecifically to polymerize proteins and other cellular components into the amorphous dark pigments known as melanin (Stevens & Davelaar, 1996; Thygesen et al., 1994).

The intimate involvement of the PPO enzyme in the browning reaction has been confirmed using several approaches. To date, the most convincing evidence comes from transgenic potato, where antisense inhibition of PPO expression resulted in greatly reduced bruising in the transgenic tubers (Bachem et al., 1994). In addition, chemical inhibitors of polyphenol oxidase activity, such as tropolone (Espin & Wichers, 1999) or kojic acid (Chen et al., 1991), inhibit the browning reaction, indicating that active PPO enzyme is required. Finally, treatment of cut tubers with antioxidant compounds delays or prevents browning; indicating that molecular oxygen is also necessary for browning (Whitaker & Lee, 1995). However, only a weak correlation between the amount of PPO activity present in tuber tissue and browning potential has been reported from potato lines exhibiting different levels of resistance to bruise (Stevens & Davelarr, 1997). Measurements of phenolic substrate levels in tubers from these same lines have revealed a stronger correlation between substrate level and browning potential or bruise susceptibility (Corsini et al., 1992; Dean et al., 1993; Sabba & Dean, 1994; Stevens & Davelarr, 1997). Taken *in toto*, these observations indicate that PPO activity is necessary, but not sufficient, for the browning reaction to occur and that most cultivars and advanced breeding lines of potato contain enough PPO activity to catalyze the browning reaction, (provided that sufficient phenolic substrate and oxygen are present).

Several potential approaches to breeding for bruise resistance in cultivated potato are apparent from these observations: 1) reducing the level of PPO enzyme activity; 2) decreasing endogenous phenolic substrate concentrations; 3) increasing the level of antioxidant compounds; and 4) improving the structural resistance to cellular damage (McGarry et al., 1996; Stevens & Davelarr, 1997). Although a transgenic approach to reducing PPO activity has been demonstrated to be effective in increasing bruise resistance, (Bachem et al., 1994) the current lack of consumer acceptance of GMO foods makes this approach impractical. Conventional breeding for low PPO levels is hampered by both the difficulty in assaying for this trait directly and the low genetic variability in PPO activity level found in elite lines of *S. tuberosum* (Stevens & Davelarr, 1997). Similarly, increasing the mechanical strength of tubers or providing antioxidants seem to be biologically implausible strategies since they require the manipulation of complex traits that are both difficult to screen for and are known to exhibit significant genotype-by-environment interactions (McGarry et al., 1996). Finally, although it is difficult to directly screen for reduced phenolic substrate levels in a breeding program, this trait has been reported to be associated with bruise resistance in several lines of cultivated potato (Corsini et al., 1992; Dean et al., 1993; Mondy & Munshi, 1993; Stevens & Davelarr, 1997).

Introgression of the low browning trait from wild *Solanum* species is one potential approach to overcoming the low variation in tuber bruise resistance and PPO levels found in *S. tuberosum* breeding lines. Several accessions of *Solanum hjertingii*, a wild disomic allotetraploid species from northern Mexico, possess a very high level of resistance to bruising (Woodwards & Jackson, 1985; Gubb et al., 1989) and have been reported to have low endogenous levels of PPO activity (Brown et al., 1999; Gubb et al., 1989; Sim et al., 1997). However, although *S. hjertingii* and *S. tuberosum* are both tetraploids, direct crosses between these species are difficult to achieve due to differences in endosperm balance number (EBN = 2 and EBN = 4 respectively (Johnston & Hanneman, 1982)). Several methods of overcoming EBN incompatibility problems have been described, including: 1) fertilization with unreduced gametes from diploid species (Den Nijs and Peloquin 1977); 2) fertilization with 2n gametes from triploid hybrids (Adiwilaga & Brown,

Table 1. Description of germplasm used in this study

Identity	Description	Bruise[1]	Ploidy	EBN[2]	Origin
hjt	*Solanum hjertingii* 251065.1	R	4X	2	Clone of PI 251065 US Potato Intro Station (NR6) USDA/ARS Sturgeon Bay, WI. (Clonal selection performed at USDA/ARS Prosser, WA)
91E22	*S. phureja* × *S. stenotomum*	S	2X	2	Clonal selection[3] USDA/ARS, Prosser, WA
95H3.3	hjt × 91E22	R	3X	–	Single seed clone (sterile) USDA/ARS Prosser, WA
D26	doubled 95H3.3	R	6X	4	Chromosome doubled 95H3.3 USDA/ARS, Prosser, WA
Lemhi Russet	*S. tuberosum*	S	4X	4	USDA/ARS, Aberdeen, ID
Ranger Russet	*S. tuberosum*	S	4X	4	USDA/ARS, Aberdeen, ID

[1] Reaction to impact bruising; R = resistant, S = susceptible.
[2] Endosperm balance number.
[3] Clonal selection from a population of *S. phureja* × *S. stenotomum* derived by recurrent selection for adaptation, (population provided by F.J. Haynes, formerly of the Dept. of Horticulture at North Carolina State University).

1991); 3) crossing with dihaploid *S. tuberosum* and embryo rescue (Watanabe et al., 1992); 4) protoplast fusion with dihaploid *S. tuberosum* (Rokka et al., 1998); and 5) utilizing the rare viable seeds produced in inter-EBN crosses (Janssen et al., 1997). This paper describes a new approach that utilizes a cross between *S. hjertingii* and a diploid 2EBN bridging species, followed by somatic chromosome doubling of the triploid progeny to produce a hexaploid 4EBN clone amenable to crossing with tetraploid *S. tuberosum*. We report the successful use of this method to introgress the low browning trait from *S. hjertingii* into *S. tuberosum*. In addition, the pattern of variation for the low browning trait observed in the BC_1 populations, and the impact this may have on further breeding progress will be described.

Materials and methods

Plant growth

The origin and descriptions of the genotypes used in these experiments are detailed in Table 1. The plants used in this study were grown from either true seed or from micro-propagated clonal plantlets maintained on modified MS without hormones (Huang & Murishige, 1976). For tuber production, seedlings or rooted plantlets were transplanted into 10-inch pots containing Sunshine Mix No. 1 potting soil (SunGro Horticulture, Bellvue, WA) and grown in a greenhouse under an 8 h light/16 h dark artificial light regime until maturity.

1st generation bridging crosses

The starting material for this study was derived from the initial crosses of *S. hjertingii* (PI251065; clonal selection 251065.1) with the diploid bridging clone 91E22 as described in Brown et al. 1999, (Figure 1A). Briefly, pollen from the diploid 91E22 male parent (a bruise susceptible clonal selection from a population of *S. phureja* × *S. stenotomum* derived by recurrent selection for adaptation. Population provided by F.J. Haynes, formerly of the Dept. of Horticulture at North Carolina State University), was used to fertilize emasculated flowers of a clonal selection of the *S. hjertingii* pistillate parent (251065.1). The F_1 seeds were germinated on half-strength modified MS without hormones (Huang & Murishige, 1976) and rooted explants from individual seed progeny were grown as described above. Tubers grown from these F_1 plants were harvested at maturity and stored at 4 °C prior to characterization for browning potential and PPO activity as described below (Brown et al., 1999). The 10 triploid progeny clones derived from these tetraploid by diploid crosses exhibited a strong degree of dominance (d/a), (Falconer, 1989), for both reduced browning potential (d/a = –1.29) and low PPO activity (d/a = –0.88) (Brown et al., 1999). A single triploid progeny clone (95H3.3) exhibiting low browning po-

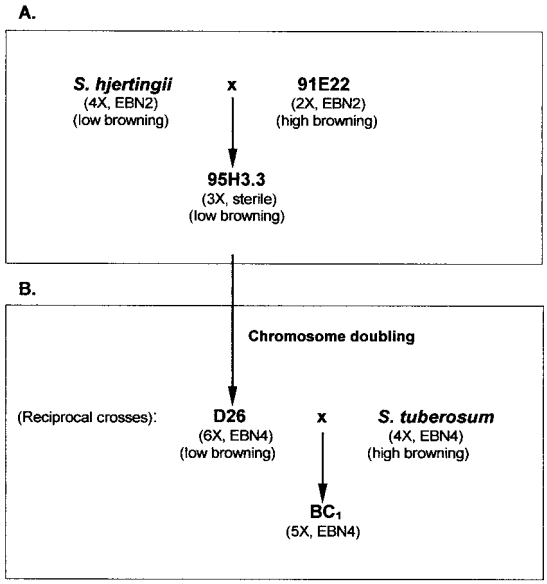

Figure 1. Breeding strategy used to introgress blackspot bruise resistance from *S. hjertingii* into cultivated *S. tuberosum*. A. First generation bridging cross between tetraploid *S. hjertingii* (251065.1) and diploid 91E22 (clonal selection from a *S. phureja* × *S. stenotomum* population). B. Chromosome doubling of the triploid bridging cross progeny (95H3.3) and second-generation introgression cross between the resulting hexaploid clone (D26) and cultivated potato (*S. tuberosum*).

tential (24% of the high parent value) and reduced PPO activity (11% of the high parent value) was selected from this cross for *in vitro* chromosome doubling and further crosses.

Somatic chromosome doubling

Callus tissue was induced from internodes of micropropagated 95H3.3 tissue and plantlets regenerated as described in Brown et al., 1991. Briefly, after two days on callus induction media (MS containing 1 mg-L^{-1} zeatin riboside and 2 mg-L^{-1} naphthalene acetic acid), the internodes were transferred to shoot induction media (MS containing 2.5 mg-L^{-1} zeatin riboside, 0.02 mg-L^{-1} naphthalene acetic acid and 0.1 mg-L^{-1} GA_3). After approximately 4 weeks, individual regenerated shoots were excised, rooted in half-strength MS medium (Brown et al., 1991) and transplanted into potting soil. At flowering, potentially hexaploid plants derived from spontaneous somatic chromosome doubling of 95H3.3 were selected based on increased leaf area and anther morphology. Chromosome doubling was confirmed by staining the pollen with 1% acetocarmine to verify the presence of large stainable pollen (Bamberg & Hanneman, 1991). Tubers harvested from these plants at maturity were evaluated for browning potential and PPO activity as described below. A single hexaploid clone (D26) exhibiting low browning potential and low PPO activity was selected for further crosses with cultivated *S. tuberosum*.

2^{nd} generation introgression crosses

Rooted explants from micro-propagated tetraploid *S. tuberosum* cultivars Lemhi Russet and Ranger Russet were grown as described above and crossed with hexaploid D26, (Figure 1B). Reciprocal crosses were made to allow evaluation of possible maternal effects. Self-pollinations of each parental clone and the tetraploid *S. hjertingii* clone 251065.1 were also made to allow evaluation of within source variability for comparison with the BC_1 progeny populations. The resulting berries were harvested approximately 6 weeks after pollination and the seeds were expressed, rinsed and air-dried. After treatment with 1500 ppm GA_3 as described previously, the seeds were planted directly into potting soil in the greenhouse. When approximately 3 cm high, the seedlings were transplanted into individual 10-inch pots and grown to maturity in the greenhouse. A randomized complete block design was used, consisting of a total of 30 plants from each self-pollination (Lemhi Russet, Ranger Russet, D26 and 251065.1) and 30 individual seed progeny plants of each reciprocal hexaploid by tetraploid BC_1 cross, (D26 × Lemhi, Lemhi × D26, D26 × Ranger, Ranger × D26) for a total of 240 plants, (each representing an experimental unit), divided randomly into 8 replicate blocks. A minimum of 15 plants of each parental clone (251065.1, 91E22, 95H3.3, D26, Lemhi Russet and Ranger Russet) were grown from tissue culture for comparison with the seed progeny. Each pot was watered with an automated drip irrigation system and received a single application of Osmocote 19: 6: 12 controlled release fertilizer (Grace Sierra, Milpitas, CA) at transplanting. Tubers were harvested by individual pot at maturity (as determined by foliar senescence; approximately 130 days after transplanting). The tubers were then stored at 6 °C for 3–7 weeks prior to characterization for browning potential and PPO activity as described below.

Chromosome counts

For confirmation of ploidy, root tips harvested from clonal *S. hjertingii* (251065.1), 91E22, 95H3.3, D26 and *S. tuberosum* (cv. Ranger Russet) were soaked in

2 mm 8-hydroxyquinoline for 5 hrs and then fixed overnight in Farmer's solution (3: 1 ethanol: acetic acid) before transfer to 70% ethanol for storage. Staining with aceto-carmine and chromosome counts were performed as described in Brown, 1988.

Browning potential assay

The total biochemical potential for tuber browning was determined using a modification of the method described in Dean et al., 1993. Triplicate sub-samples of two tubers each were taken from each pot, (representing a total of six tubers per pot). Each tuber was finely chopped and the tissue from the two tubers per replicate was pooled. A 5-gram subsample of each pooled two-tuber replicate was weighed out, mixed with 10 mls of cold extraction buffer (0.05 M $NaPO_4$ pH 6.5) and homogenized at high speed in a 50 ml stainless steel Waring blender cup for 30 seconds. The resulting slurry was filtered through Whatman #1 filter paper and a 100 μl aliquot was immediately removed and diluted for the PPO assay described below. A standard 3 ml volume of the remaining extract was allowed to oxidize in a 18mm $\times$ 100 mm glass tube at room temperature for 18–24 hours. The oxidized samples were then vortexed and 1.5 ml aliquots were centrifuged at 12,000 g for 10 minutes. Browning potential was expressed as the absorbance of the clarified sample measured at 475 nm using a Shimadzu model UV160U spectrophotometer. Samples with an absorbance of greater than 0.800 OD units were diluted with extraction buffer to bring them into the linear range for the spectrophotometer.

Polyphenol oxidase enzyme activity assay

PPO enzyme activity was determined using a modification of the method described in Brown et al., 1999. Immediately after homogenizing and filtering the tuber extracts for the browning potential assay described above, a 1: 5 dilution was made by diluting a 100μl subsample of the filtrate into 400 μl of cold assay buffer (0.05M $NaPO_4$ pH 6.8). The diluted samples were placed on ice and the PPO catecholase activity present in the extracts were determined by placing a 50 μl aliquot of the diluted extract into a 1.5 ml polystyrene cuvette and vigorously injecting 1.450 mls of 50mM catechol (Sigma C9510) in room temperature assay buffer. The cuvette was immediately placed into a Shimadzu model UV160U spectrophotometer and the absorbance at 400 nm was measured at 2-second intervals for 20 seconds. The slope and

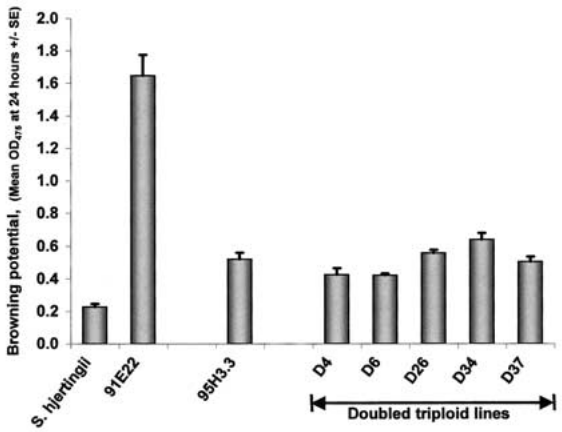

Figure 2. Browning potential results from the first generation bridging cross and the clonal selections derived from somatic chromosome doubling of the resulting triploid clone (95H3.3). Browning potential is expressed as the mean OD_{475} of the oxidized tuber extracts from 5 replicate samples of each clonal selection.

Pearson's correlation coefficient for the initial linear portion of the increase in absorbance was determined using the linear least squares analysis functions SLOPE and RSQ from the Microsoft Excel program. A unit of PPO activity was defined as an increase of one OD_{400} unit/min/gram fresh weight.

Statistical analyses

Progress towards reduced browning potential or reduced PPO activity in the introgressed populations was analyzed using the MIXED procedure from the SAS software package, (Copyright, SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA), to perform ANOVA and contrast analyses after confirming the normality and homoscedastisity of the data. (PROC MIXED tests both fixed effects and variance/covariance components using iterative optimization of a likelihood function (Newton-Raphson method)). The Pearson correlation coefficients for the relationship between PPO activity and browning potential within individual crosses were calculated using the CORR procedure from SAS. The Pearson correlation coefficient was also calculated for the mean PPO activity expressed as activity intervals versus the mean browning potential using the RSQ function from the Microsoft Excel program.

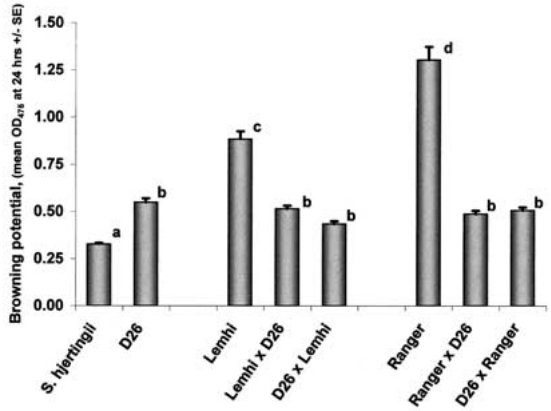

Figure 3. Browning potential data from the progeny of the second-generation introgression crosses and from the parental self-seed progeny populations. Results from the self-seed progeny from *S. hjertingii* (251065.1 self) are included for comparison. Browning potential is expressed as the mean OD_{475} of the oxidized tuber extracts from at least 28 replicate samples of each cross or self. Means not sharing the same letter are significantly different at the P = 0.01 confidence level.

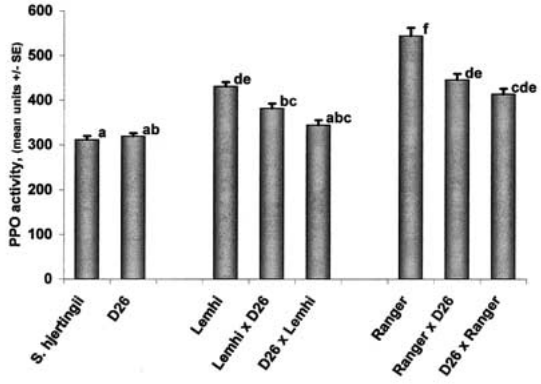

Figure 4. Polyphenol oxidase (PPO) enzyme activity results from the progeny of second-generation introgression crosses and from the parental self-seed progeny populations. Results from the self-seed progeny of *S. hjertingii* (251065.1 self) are included for comparison. PPO activity is expressed as the catecholase activity (change in OD_{400}/min/gfw.) in extracts from at least 28 replicate samples of each cross or self. Means not sharing the same letter are significantly different at the P = 0.01 confidence level.

Results

From 50 independent clones regenerated after callus induction, five doubled clones derived from 95H3.3 were screened for browning potential. The ploidy of the triploid 95H3.3 and hexaploid D26 clones were confirmed through chromosome counts of root tip squashes. Expression of the low browning trait was found to be very similar to the triploid 95H3.3 in all five doubled clones (Figure 2). A single hexaploid clone (D26) was selected for crossing with cultivated varieties of *S. tuberosum* based on low browning potential, (35% of the high parent), and good pollen production.

Crosses of D26 with the bruise-prone potato cultivars Lemhi Russet and Ranger Russet (Figure 1B) were successful and produced berries containing an average of 22.8 and 25.5 presumably pentaploid seeds per berry for the Ranger Russet and Lemhi Russet crosses respectively.

An analysis of variance of the browning potential results from the control and the BC_1 tubers indicated a highly significant overall genotype effect for browning potential. Further orthogonal contrast analyses revealed that the seed progeny from the crosses of D26 with *S. tuberosum* produced tubers expressing a significantly lower potential for browning than the seed progeny from the selfed *S. tuberosum* parents (Figure 3). No significant difference in browning potential was observed between reciprocal crosses of D26 and either *S. tuberosum* parent and, therefore, the data from reciprocal crosses were combined for further analyses. The average browning potential value for the pooled data from the crosses of D26 with Lemhi Russet was 0.474 compared to 0.883 for the Lemhi Russet self population, and 0.497 for the Ranger crosses compared to 1.304 for the Ranger selfs. No significant differences in browning potential were observed in comparisons between the D26 self-seed progeny tubers and the crosses of D26 with either Lemhi or Ranger, again indicating a high degree of dominance for the low browning trait.

Analysis of variance of the PPO activity data also indicated a highly significant genotype effect for PPO level. Subsequent contrast analyses indicated a significant reduction of PPO activity levels in the BC_1 progeny relative to the *S. tuberosum* parents. No significant difference in PPO activity was observed between reciprocal crosses of D26 with either*S. tuberosum* parent and the data from the reciprocal crosses were again combined for further analyses. Pooled reciprocal data yielded an average of 362 units of PPO activity for the Lemhi Russet BC_1 progeny compared to 431 units for the Lemhi self-seed population, and 430 units for the Ranger Russet BC_1 progeny compared to 545 units for the Ranger selfs. Tubers from the crosses of D26 with either Lemhi or Ranger exhibited significantly lower PPO activity than tubers from the correspond-

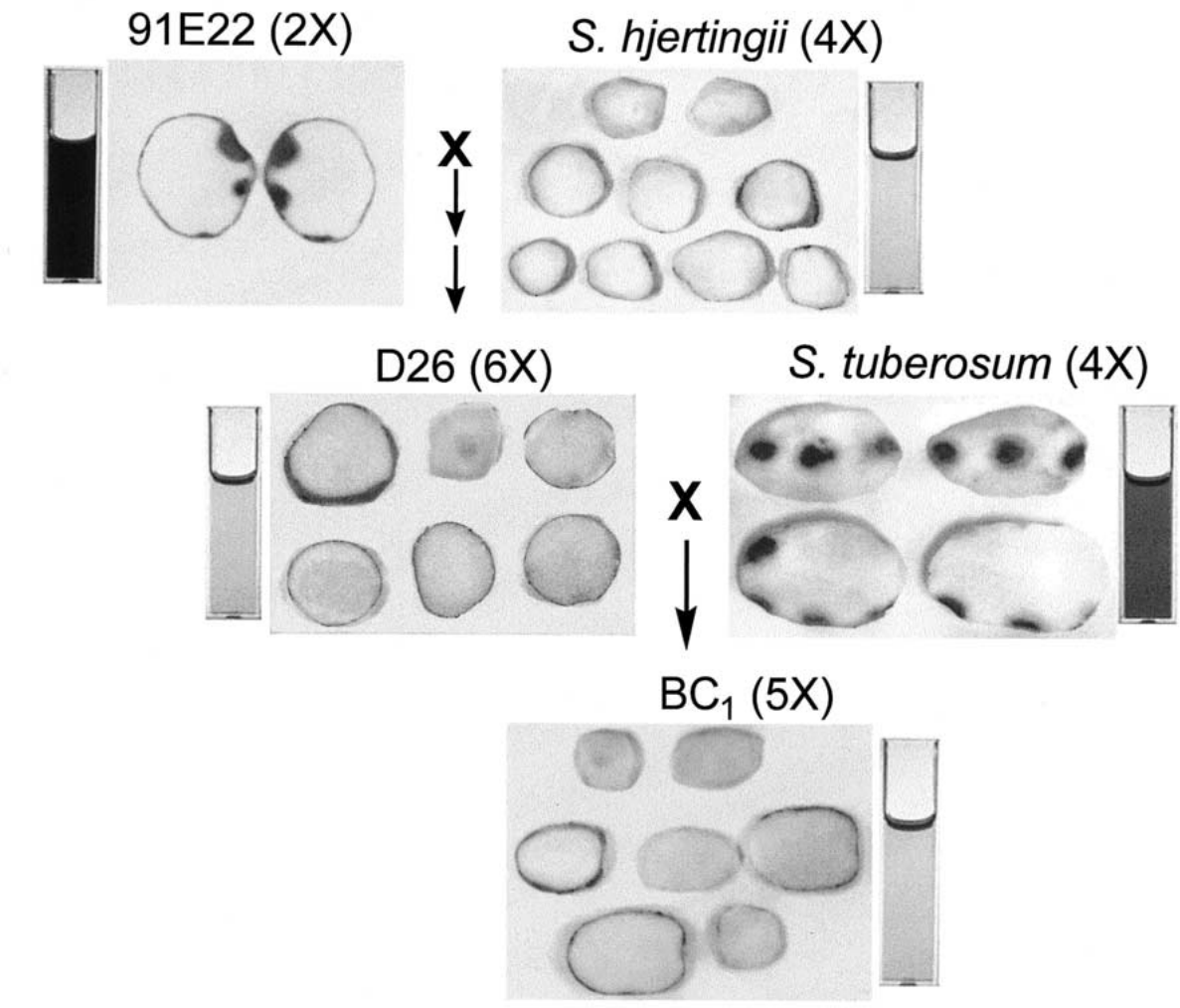

Figure 5. Correspondence between progress towards reduced browning potential and reduced bruise susceptibility in tubers from the various crosses reported in this paper. Representative tubers 10 days after impact damage are paired with cuvettes containing oxidized tuber extracts used to determine tuber browning potential for these same clones.

ing *S. tuberosum* parent population. In contrast to the browning potential results, the PPO activity from both the Ranger and Lemhi crosses were intermediate between the two parents (Figure 4). However, while the difference between the Ranger crosses and the D26 population was significant at the $p < 0.001$ level, the difference for the Lemhi crosses was only significant at the $0.01 < p < 0.05$ level, probably due to the low differential in PPO activity observed between the Lemhi and D26 parent populations. Thus, in contrast to the dominant expression pattern observed for browning potential, the pattern of expression of PPO activity in the BC_1 population is best described using a co-dominant or intermediate inheritance model.

Discussion

The reduction in browning potential we observed in both the initial tetraploid by diploid population and in the hexaploid by tetraploid BC_1 populations demonstrates that introgression of this trait from wild germplasm is a practical approach to increasing browning resistance in cultivated potato. Although we did not directly determine bruise susceptibility of tubers from

the introgressed populations, the *in vitro* browning potential assay used in this study has been shown to be a good predictor of the bruise reaction (Dean et al., 1993). This correlation was tested for the current study by subjecting a subsample of tubers to the browning potential assay used in this study and the falling weight impact assay as described in Deanet al., 1993. Results from these experiments confirm the validity of using the browning potential assay as an indicator of bruise susceptibility in these populations (data not shown). Representative results from these experiments are illustrated in Figure 5.

The low browning potential phenotype derived from *S. hjertingii* exhibits a dominant pattern of inheritance that was observed in both the initial cross with the high browning diploid 91E22 clone and in the second generation crosses with cultivated *S. tuberosum*. Degree of dominance (d/a) is a concept developed to give a numerical value to the tendency for the mean phenotypic values of the progeny from a cross to deviate from the mid-parent value (Falconer, 1989):

d/a) = (Progeny mean – Mid-parent values) ÷
(Mid-parent value – Low parent mean)

Greater phenotypic dominance is indicated by increasing deviation of d/a from zero, with negative and positive values indicating dominance of the low and high parent respectively and values exceeding 1 or less than –1 indicating overdominance. Applying this formula to the data from our introgression experiments, the low browning potential phenotype derived from *S. hjertingii* appears to be dominant over the high browning phenotype present in both the 91E22 diploid bridging clone and the cultivated potato lines (Table 2A). In the BC_1 populations there is even some suggestion of over-dominance. In contrast, the degree of dominance values for PPO activity in these crosses indicates a more co-dominant or intermediate inheritance pattern (Table 2B).

Because the PPO enzyme is expressed from a nuclear encoded gene family and then localized to plastids during translation (Mayer, 1987; Thygesen et al., 1994), plastid associated mechanisms having an effect on the uptake, conformation, stability or availability of the PPO enzyme would be likely to show significant maternal effects. Previous studies on inheritance of the low browning trait from *S. hjertingii* used this species as the female parent and therefore maternal effects remained a possibility (Brown et al., 1999; Gubb et al., 1989; Woodwards and Jackson 1985). Comparison of the BC_1 reciprocal crosses revealed no significant difference for either browning potential or PPO activity, thus demonstrating the absence of a maternal effect for these traits (Data not shown).

The apparent independence of browning potential and PPO activity is illustrated by a comparison of the 91E22 and D26 results. While the progeny lines from these two clones yielded extremely divergent browning potential averages (1.788 and 0.548 respectively), they contained approximately equal amounts of PPO activity in the tuber extracts (averaging 321.6 and 318.7 units respectively). In addition, graphs of mean browning potential versus PPO activity for the BC_1 populations and parental lines make it apparent that the progress towards decreased browning potential we observed in these populations did not necessarily involve a correspondingly large decrease in PPO activity (Figure 6).

These results, taken alone, suggest that the initial level of PPO activity may not be the primary mechanism responsible for the low browning trait derived from *S. hjertingii*. However, in contrast to previous reports, our data indicates a weak but statistically significant correlation between browning potential and PPO activity levels analyzed over all genotypes, (Pearson's correlation coefficient of 0.45 with a $p < 0.0001$). This correlation is particularly apparent if mean PPO activity intervals are used rather than the raw values (Pearson's correlation coefficient of 0.928; Figure 7). Our ability to detect a significant correlation that was not apparent in previous studies most likely stems from the large range of browning potential and PPO values represented in the populations we produced in this study, (ranging from an OD_{475} of 0.215 to 2.832 for browning potential and from 170 to 1109 units for PPO activity).

A comparison of the PPO assay methods used in this and in previous studies further suggests that decreased PPO activity may indeed be playing an important role in the browning potential reduction observed in the BC_1 populations. The current study utilized a kinetic assay that measures PPO activity as the slope of the absorbance increase over time during the initial linear portion of the increase, usually lasting less than 30 seconds. In contrast, previous studies reporting low or absent PPO activity in *S. hjertingii* used an end-point assay that calculated the activity based on the difference in absorbance measured at two relatively widely separated time points (Brown et al., 1999; Mapson et al., 1963; Stark et al., 1985; Vertregt, 1968; Weaver et al., 1970). The contrasting results

Table 2. Degree of dominance for progeny means of browning potential and PPO activity in the 1st generation bridging cross between *S. hjertingii* and the diploid 91E22 parent, and the 2nd generation introgression crosses between the D26 hybrid and *S. tuberosum*

A. Degree of dominance for browning potential (OD475 at 24 hrs)				
Low parent	High parent	Mid-parent	Progeny	d/a
hjt	91E22		D26	
0.327	1.788	1.058	0.548	–0.697
D26	Ranger		Ranger × D26	
0.548	1.300	0.924	0.497	–1.136
D26	Lemhi		Lemhi × D26	
0.548	0.883	0.716	0.474	–1.442
B. Degree of dominance for PPO activity (change in OD400 /min/gfw)				
Low parent	High parent	Mid-parent	Progeny	d/a
hjt	91E22		D26	
311.0	321.6	316.3	318.7	0.452
D26	Ranger		Ranger × D26	
318.7	553.2	435.9	428.7	–0.061
D26	Lemhi		Lemhi × D26	
318.7	430.8	374.7	362.8	–0.214

obtained using these two types of assays suggests that the PPO activity present in *S. hjertingii* may be unstable relative to the PPO activity in the high browning parents used in these studies. A genetic mechanism derived from *S. hjertingii* that causes destabilization of PPO activity could explain the dominant inheritance pattern for low browning potential observed in crosses of high browning clones with *S. hjertingii*. However, although these results are extremely interesting from a mechanistic standpoint, it is important to note that PPO activity is generally a poor predictor of bruising in individual plants, as low browning potential is sometimes associated with high PPO activity and *vice versa*. Therefore, the correlation between browning potential and PPO activity is likely to be of questionable value in a breeding program.

Additional mechanisms can be hypothesized that would account for the dominant inheritance pattern of decreased browning potential without directly influencing the level of the PPO enzyme itself. For example, reduced levels of substrate are capable of reducing browning potential and could arise from either the presence of a pathway that converts the normal PPO substrate(s) into a non-substrate for pigment formation, or from the production of an inhibitor of the pathway normally producing the substrate(s). A dominant inheritance pattern would also be observed if a pathway was present that converts the diquinone products of the PPO reaction into non-pigment forming compounds, or if an inhibitor of the PPO enzyme were present in *S. hjertingii*. The populations we have produced will be extremely useful in studying the biochemical mechanism behind the low browning trait derived from *S. hjertingii.* For example, determining enzyme kinetics, substrate affinities, enzyme stability and substrate levels in BC_1 progeny tubers and comparing the results to the parental tubers will assist in determining which, if any, of the potential mechanisms outlined above are active in this system.

Bruising is known to be a complex trait and the relative importance of the many factors influencing bruise susceptibility is still subject to debate (McGarry et al., 1996). However our initial effort to introgress the low browning trait from *S. hjertingii* into potato breeding populations has been successful. The results we obtained demonstrate that further work with these populations may result in a significant reduction in blackspot bruise losses in cultivated potato.

Note

This work represents a cooperative investigation between the ARS/USDA, and the Washington State University Agricultural Research Center, Prosser, WA 99350.

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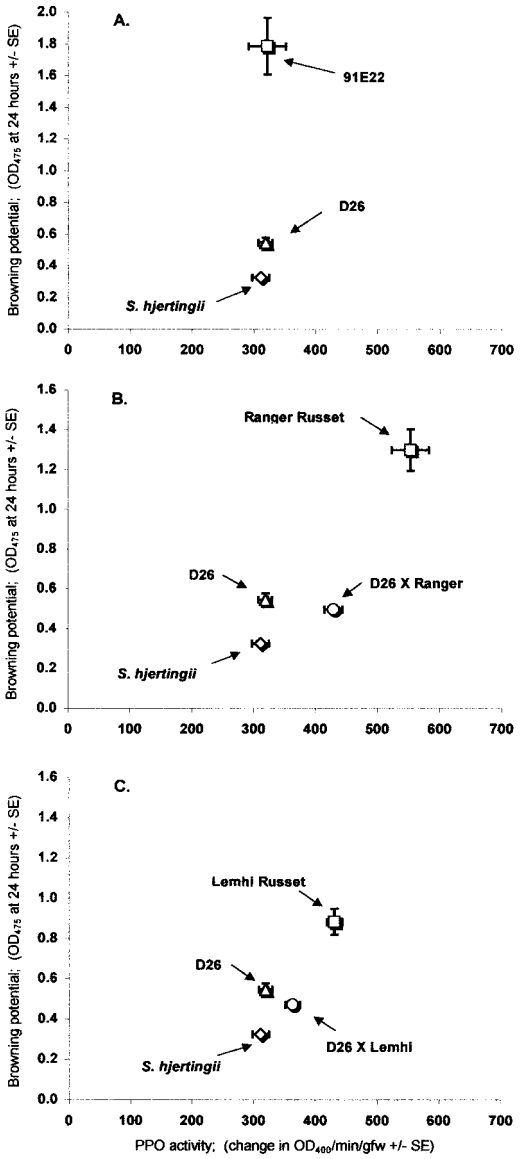

Figure 6. Comparison of average browning potential and PPO activity levels in tubers from the progeny of the crosses reported in this paper. A. Results from clones of the first generation bridging cross and parental lines. (Mean of 5 replicates) B. & C. Results from the second generation introgression cross between hexaploid hybrid D26 and Ranger Russet (B.) or Lemhi Russet (C.), (60 replicates from pooled reciprocal crosses for each parent), and parental self-seed progeny populations, (minimum of 26 replicates). Results from the self-seed progeny *S. hjertingii* (251065.1 self; 30 replicates) are included for comparison.

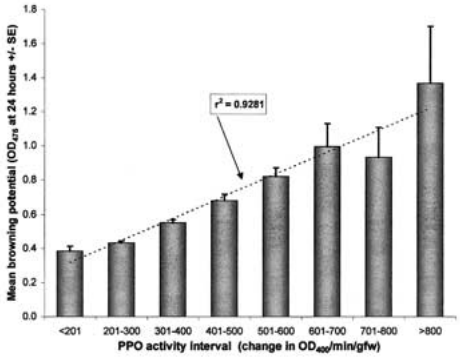

Figure 7. Correlation between browning potential and polyphenol oxidase activity interval using the mean browning potential values from all progeny of the second generation introgression crosses and from the parental self-seed progeny populations. Sample population consists of results from triplicate measurements of browning potential and PPO activity in tuber extracts from 228 clones. The best-fit linear least squares determination (dotted line) and Pearson's correlation coefficient (r^2) were determined using Microsoft Excel 2000.

product by the USDA and does not imply its approval to the exclusion of other products that may be suitable. This article reports the results of research only.

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